



Review

Biopolymer Matrices Based on Chitosan and Fibroin: A Review Focused on Methods for Studying Surface Properties

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Abstract: For the creation of tissue-engineered structures based on natural biopolymers with the necessary chemical, physical, adhesive, morphological, and regenerative properties, biocompatible materials based on polysaccharides and proteins are used. This work is devoted to a problem of the technology of polymeric materials for biomedical purposes: the creation of biopolymer tissue engineering matrix and the development of a methodology for studying morphology and functional properties of their surface to establish the prospects for using the material for contact with living objects. The conditions for the formation of scaffolds based on composite materials of chitosan and fibroin determine the structure of the material, the thickness and orientation of molecular layers, the surface morphology, and other parameters that affect cell adhesion and growth. The analysis of studies of the morphology and properties of the surface of biopolymer matrices obtained using different methods of molding from solutions of chitosan and fibroin is carried out.

Keywords: biopolymers; chitosan; fibroin; tissue engineering; scaffolds; surface morphology; adhesion; free surface energy; AFM; modulation interference microscope (MIM)



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1. Introduction

Modern technologies make it possible to develop composite materials based on biopolymers with unique desired properties obtained as a result of modification: bioreactivity and biodegradation in natural environmental conditions, bioinertness in the environment of a living organism, resistance to mechanical stress, etc. [1,2].

Biopolymers are actively used in scientific developments aimed at obtaining medical and biological products with specified and controlled properties [3–5]. One of the promising areas of such research is tissue engineering, the purpose of which is to develop methods for the regeneration of lost or damaged tissues, as well as entire organs, using new biomaterials and cellular technologies. The critical issue of tissue engineering is the creation of a suitable artificial biomimetic matrix—a scaffold for targeted tissue regeneration, providing conditions for cell growth and, ultimately, their integration with the surrounding tissue. [6]. Biomaterials can form matrices and scaffolds of various types (Figure 1) [7], which provide structural and functional support of cells on their surface, and their components regulate cell migration and proliferation [8], thereby affecting tissue formation, blood coagulation, adsorption of biomacromolecules and wound healing [9].

The most common basis for creating materials capable of maintaining and regulating moisture, as well as adsorbing proteases from wound exudate, are proteins and polysaccharides (hyaluronic acid, alginates, starch, carrageenans, collagen, gelatin, dextran, polyhydroxyalkanoates, chitosan and fibroin) [10–15], many of which have antioxidant, antimicrobial and anti-inflammatory properties, reduce the risk of inflammation, and are highly effective biostimulants.

The surface activity of polysaccharides and proteins is able to regulate the enzymatic expression and optimal moisture content for wound healing while ensuring natural drainage [16,17]. Scaffolds based on these compounds provide the structural integrity

of tissue structures, control the delivery of drugs and proteins to tissues, and serve as adhesives or barriers between tissue and various material surfaces [18]. In the process of biodegradation, they are split into simple compounds that can take an active part in metabolism at the cellular level or are excreted from the body [19].

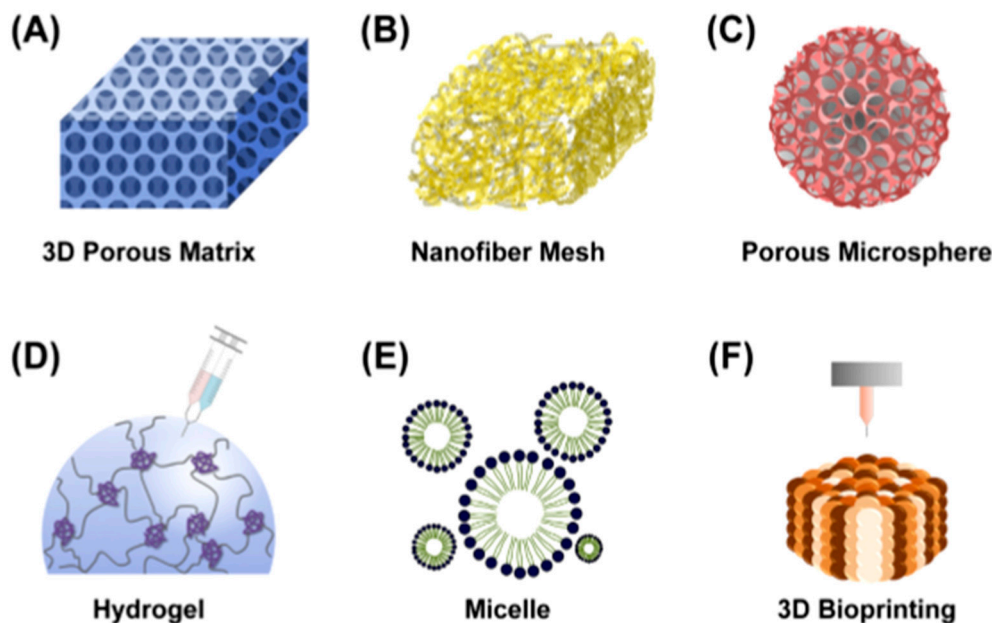


Figure 1. Different types of polymeric scaffolds for tissue engineering: (A) 3D porous matrix, (B) nanofibrous mesh, (C) porous microsphere, (D) hydrogel, (E) micelle and (F) 3D bioprinting [7].

Considering the unique properties—biocompatibility, gel-forming and mucoadhesive properties—and the ability to complex formation, and based on the quantitative analysis of publications devoted to the development of biopolymer matrices for tissue engineering, the most promising biopolymers for the development of a carrier support for attachment and growth of cells should include chitosan and silk fibroin [20–24]. Chitosan promotes tissue adhesion and regeneration due to its structural similarity with glycosaminoglycans, which are part of the extracellular matrix, and the structural protein silk fibroin is an excellent substrate for cell attachment and proliferation. Fibroin and chitosan, as well as composite materials based on them, provide the structural integrity of tissue structures, control the delivery of growth factors and enzymes to tissues and cell cultures, and support adhesion and proliferation and the functional activity of living cytoobjects.

To date, there are many methods for obtaining biopolymer matrices—casting films [25–27], spin-coating [27–30] or cryotropic gelation [31,32], electrospinning [33–35]. The method of electrospinning is widely known in tissue engineering, regenerative medicine, dentistry and matrices based on biopolymers, and compositions based on them have a number of unique properties that are distinguished by a number of authors, including Prof. Zafar [36,37].

Using the above methods, materials are obtained that differ in internal structure, pore size and mechanical properties of the surface [38], and morphology. The morphology of the surface of the biopolymer matrix, namely its roughness, is a very important parameter affecting the surface wettability, protein adsorption, cell attachment and further cell growth, as well as the course of the inflammatory reaction. Thus, the methodology for assessing the surface properties of biopolymer materials intended for use as matrices for tissue engineering is an important part of the research in the technology of biomedical materials.

The purpose of this review is to analyze research methods and study morphology and functional properties of biopolymer matrices based on chitosan and fibroin with different types of surfaces obtained from solutions of biopolymers.

2. Existing Directions and Methods of Obtaining Porous Biopolymer Matrix

Porous structures with a system of large interpenetrating pores can be obtained by freezing polysaccharide solutions containing a crosslinking reagent and lyophilizing a polymer hydrogel [39–41]. In this case, the characteristics of the initial system (molecular weight of the polymer, concentration, content of the cross-linking reagent) determine the structure of the resulting matrix (the nature and distribution of pores by size), as well as the degree of adhesion and spreading of cells, which, in turn, determine their subsequent growth and proliferation. A modification of this method is possible: a polymer solution is lyophilized, followed by the transformation of the material into an insoluble form by chemical crosslinking of macromolecules already in the solid phase. In this case, wide-porous spongy materials are obtained, which are called cryostructures [42]. Currently, the electrospinning method is used to obtain fibrous mats consisting of sub-microfibers and nanofibers. Electrostatic forces are applied to the polymer solution from a jet, which, as the solvent evaporates, transforms into nanofibers and solidifies on the manifold. In recent years, the method of electrospinning nonwoven fibrous materials has been developed mainly for the processing of natural polymers [33,36,37], since materials based on them are characterized by biocompatibility and biodegradability in the body, and a fibrous surface with nano-sized structural elements promotes cell attachment and the formation of living tissues. Nanofibers derived from biopolymers activate fibroblasts, which migrate to the dermal layer, which releases the main components of the extracellular matrix to heal damaged tissue. The production of nanofibrous materials for various purposes, including scaffolds for tissue engineering, based on chitosan and, in particular, silk fibroin, by the method of electrospinning, was described in detail in the works of prof. Kaplan and other researchers, including prof. Zhang [43–55].

Another promising method for forming matrices from solutions of biopolymers for tissue engineering and regenerative medicine is the production of mono- and multilayer nano- and micro-polymer film coatings by the spin-coating method. A spinning coating allows one to obtain uniform thin films and coatings of varying thickness on a flat substrate using a special device—a spin-coater. The spin-coating process involves the application of the required volume of the polymer solution to the center of the substrate, and then acceleration and rotation of the substrate at high speed (3000–8000 rpm). The final coating thickness depends on the viscosity and nature of the initial solution and solvent, as well as on the parameters of centrifugation and temperature [56,57].

This method is used in developments related to tissue engineering due to the convenience of studying cell adhesion on a micro- and nanoscale surface (with known morpho-functional), proliferation, cytotoxicity when obtaining mono- and multilayer biopolymer coatings with desired sorption, hemostatic, antibacterial and hygienic properties [58–61].

Conventional films are produced by casting followed by solvent evaporation. These methods make it possible to include biologically active compounds, enzymes, and other protein compounds in their structure [62,63]. For improve the water-resistance and mechanical properties of proteins and polysaccharides, are used chemical crosslinking methods. Most of all, chitosan is suitable for preparing materials using cross-linking reagents, because of its amino groups, which are more reactive than the hydroxyl groups of other polysaccharides.

3. Preparation Biomaterials from the Chitosan Solutions

Chitosan is a polycationic biopolymer, a linear deacetylated polysaccharide derivative of natural-origin chitin. It is known that it consists of 2-acetamido-2-deoxy- β -D-glucose via a β (1 \rightarrow 4) bond [64]. Chitosan can be characterized by the degree of deacetylation and molecular weight; these parameters can differ significantly, which affects the properties of biopolymer materials and their area of use. The influence of the different degrees of deacetylation and molecular weight of chitosan on the hydrophilicity, degradation, mechanical properties and biocompatibility of chitosan films was evaluated in [65]. The results showed that the degree of deacetylation affects the hydrophilicity and biocompatibility of

the chitosan films. Reference [66] discusses the effects of molecular weight and degree of deacetylation cytotoxicity of chitosan molecules and nanoparticles. Chitosan molecules and nanoparticles exhibited comparable cytotoxicity against the A549 cells. Cytotoxicity of both chitosan entities was attenuated by decreasing polymer DD but was less affected by a decrease in Mw. The molecular weight, on the other hand, affected the rate of degradation and the mechanical properties. Chitosan, with a higher degree of deacetylation and molecular weight, was more suitable for tissue engineering applications [65]; these data are confirmed in Reference [67], which showed that activated fibroblasts appeared more in the higher deacetylation degree of chitosan.

The degree of deacetylation determines the fraction of free amino groups, which are much more reactive than the acetamide groups of chitin. The presence of an amino group in the anhydropyranose monomer unit ensures the solubility of chitosan in aqueous solutions of organic acids, which is possible when the degree of deacetylation is more than 60%. Figure 2 shows the structure of the protonated form of chitosan.

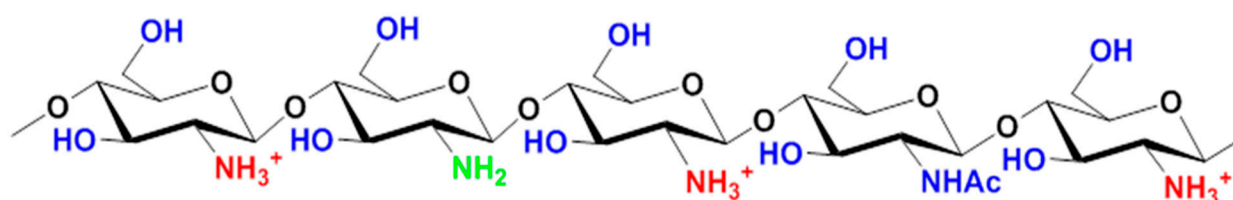


Figure 2. Chemical structure of the protonated form of chitosan.

Owing to its amino group, covalently cross-linked materials for various purposes with specified and controlled properties have been obtained [68]. Chemical crosslinking allows an irreversible transition from a chitosan solution to a hydrogel, and this technique is used in the preparation of various types of biomaterials [69–71]. The properties of chitosan hydrogels, the supramolecular and porous structure, the degree of swelling, and mechanical strength can vary due to changes in the crosslinking conditions [72,73].

Some cross-linking reagents have a certain toxicity and impart it to the resulting products, limiting their applications in medicine and tissue engineering. Therefore, recently, the use of an ecologically clean, bioactive and biocompatible covalent cross-linking agent of plant origin, genipin, has become widespread [74]. It is known as a hepatoprotective agent with pronounced anti-inflammatory, antioxidant and anti-diabetic properties, and is also actively used in the treatment of various inflammatory diseases and even cancer [75]. It was found that chitosan scaffolds, sponges, fibrous materials and products based on them spatially cross-linked by genipin not only are safe for the animal organism as a whole but also have no cytotoxic effect on various cell populations [76]. The obtained materials contribute to the proliferation of cytoorganisms; it was recorded that an increase in the amount of genipin directly affects improves cell adhesion. Depending on the degree of cross-linking, cells acquire different morphology when attached to the surface of the carrier material: single with minimal contact with the carrier, cell fusion, spreading, and local adhesion formations [31,32,77].

Chitosan is soluble in water only in an acidic media, when the primary amino groups of chitosan are protonated and the macromolecule acquires a positive charge; therefore, biopolymer matrices based on chitosan can be obtained only from its solution in an aqueous solution of an organic acid. Unlike chitosan, degummed silk—fibroin—is soluble in the entire pH range, and this, along with good fiber-forming ability, expands the possibilities of obtaining porous biopolymer matrices on its basis for growing cells.

4. Application Fibroin to the Preparation of Polymer Matrices

Silk fibroin, prepared from silkworms (mulberry silkworm of the *Bombyx mori*), has become a widely used biomaterial due to its unique physicomachanical and especially strength properties and high elasticity, biocompatibility, well-studied surface chemistry,

controlled degradation, water and oxygen permeability, renewable reserves, low cost and ease of processing. The amino acid composition of *B. Mori* silk fibroin, purified from sericin, consists mainly of glycine (Gly) (43%), alanine (Ala) (30%) and serine (12%) and a small number of other amino acids [78]. A simplified fibroin formula is shown in Figure 3.

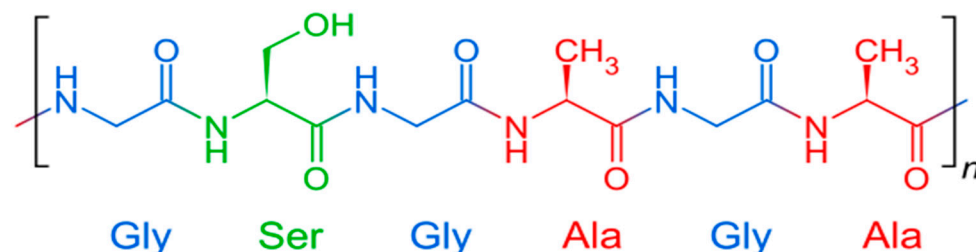


Figure 3. Chemical structure of fibroin.

Rather easy processing and processing of this raw material contribute to the production of biopolymer forms with different structures, including fibrous, film and three-dimensional (3D) porous hydrogels, complex multi-level structures (micropatterned) [79].

Films of various sizes with a variable set of parameters and characteristics can be obtained from aqueous solutions of purified fibroin. For example, Wang et al. obtained nanoscale fibroin films from aqueous solutions using a layer-by-layer technique [53]. This method makes it possible to obtain biopolymer coatings of a given size and thickness. Such nanosized films excellently support the adhesion and proliferation of mesenchymal stem cells [53]. Composite coatings obtained by Li et al., using the layer-by-layer formation of a material based on fibroin, the addition of which significantly improves biocompatibility and hydrophilicity, in combination with the polysaccharide chitosan, and with the addition of heparin, increasing the antibacterial activity to 95%, the coatings have improved biological capacity [80]. It has been shown that the attachment of fibroblasts to silk films is the same as for films based on collagen [81]. Other mammalian and insect cells also showed good adhesion to fibroin coatings as compared to collagen films [82]. Chemically modified fibroin-based films are used to improve osteoblast cell attachment and bone formation [83]. Biocompatible fibrous materials that regulate the formation of vascularized reticular connective tissue are obtained by electrospinning from fibroin [84].

5. Application of a Mix of the Chitosan with Proteins to Regulate the Morphofunctional Properties of Polymer Matrix

The use of a mix of chitosan with proteins makes it possible to purposefully regulate the physicochemical, chemical and structural parameters of the material and the morphology of the surface of products. Thus, in one study [85], it was shown that when chitosan is introduced into a collagen solution, the polymer system of the matrix undergoes a structural transition. In addition, the conditions for chemical crosslinking of the protein-containing system noticeably affect the surface and pore morphology. It has been established that the addition of chitosan increases the efficiency of cross-linking of the protein, which is involved in the spatial network of cross-linked chitosan and can increase the biostability of the scaffold [85,86]. Scaffolds made of chitosan and silk fibroin have pronounced compatibility with living cells, water-resistance and limited swelling. The homogeneous porous structure together with the proven compatibility of the two natural biopolymers provides controlled mechanical properties. By varying the amount of the system component, it is possible to control the modulus of elasticity and strength [54,87,88].

The chemical composition of the surface of polymer mixtures, morphology and adhesive properties are very important for cell adhesion, proliferation, etc. To study the proliferative activity of cells, the morphofunctional state of the surface of composite products based on chitosan and fibroin should be taken into account. It was established in [89] that a three-dimensional scaffold based on chitosan and fibroin (Figure 4) can serve as a carrier for stem cells to repair cartilage defects and may be used for cartilage tissue engi-

neering. By applying SEM and confocal microscopy, it was found that matrices based on fibroin-polysaccharide compositions promoted cell proliferation and attachment and had metabolic activity [90–92], which is due to a combination of properties: silk fibroin is a substrate for cell attachment and proliferation, and chitosan promotes tissue regeneration due to structural similarity with glycosaminoglycans, which are part of the extracellular matrix.

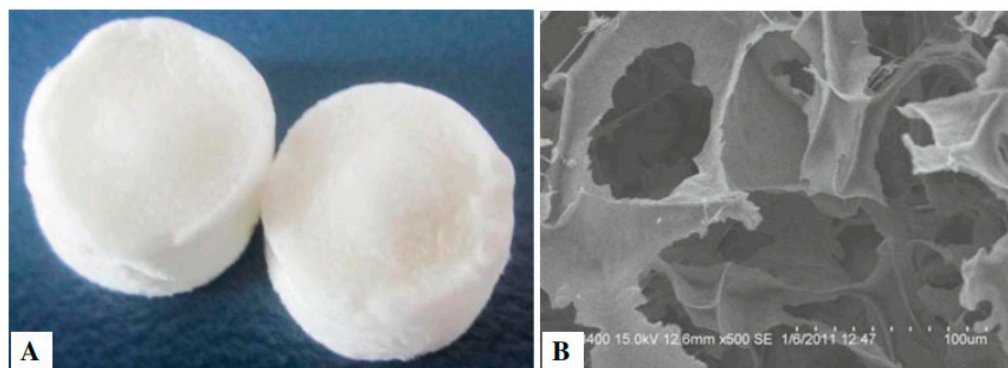


Figure 4. (A) Three-dimensional scaffold based on chitosan and fibroin. (B) is the scaffold surface obtained using a scanning electron microscope (magnification $\times 500$) [89].

6. Investigation of the Surface of Biopolymer Materials

Information about the dimensions and geometric parameters of structural elements is important for research in the development and search for ways of modification and structuring, as well as for the production of products intended for contact with the environment of a living organism. Each type of test material has its own specific surface properties. For example, the surface of polymeric materials of biological origin is more prone to irreversible damage and deformation than the surface of synthetic polymers. It has been established that polymer systems have a higher surface molecular mobility, in contrast to inorganic materials [93]. The authors of this review have considered in more detail the following three universal methods of surface research: tensiometry, atomic force microscopy (AFM) and modulation interference laser microscopy.

Surface wetting phenomena can contribute to the study of intermolecular forces and dynamics of interactions of macromolecular chains, hydrophobic and hydrophilic effects, adhesion, acidic and basic properties of the solid material surface, the ability to sorb various low molecular substances, surface physicochemistry and surface phenomena for polymer matrices and coatings of various types. Therefore, one of the most versatile, reliable and appropriate parameters for characterizing the physical and chemistry of the surface and surface topography of biopolymer coatings [94], and for studying the effect of the polymer surface and its roughness on proliferation, differentiation and cell adhesion [95–97], is the value of surface energy material obtained by tensiometry.

Today, atomic force microscopy (AFM) is known as one of the most effective methods for direct measurement of microstructure parameters and detection of intermolecular forces at the nanoscale level with atomic resolution characteristics, which expands the scope of its use [98]. The working part of the AFM is a micro-console system operating in three modes. Semi-contact mode is a key advancement in AFM, which combines the advantages of both contactless and contact modes by super-resolution and reliable data collection on soft or hard samples.

One of the most modern, reliable and super-resolving methods that is used for applied and fundamental problems of biomedical research, which makes it possible to quickly assess the morphofunctionality of biopolymer coatings, is interference microscopy [99,100]. At the heart of the modern optical method of modulation laser interference microscopy, the local phases of the modulated object of the light wave are measured and taken into account with full control of the polarization of the region, and the obtained interferograms

provided on the photodetector after mathematical processing restore the phase portrait of the portrait field of view (Figure 5) [101,102].

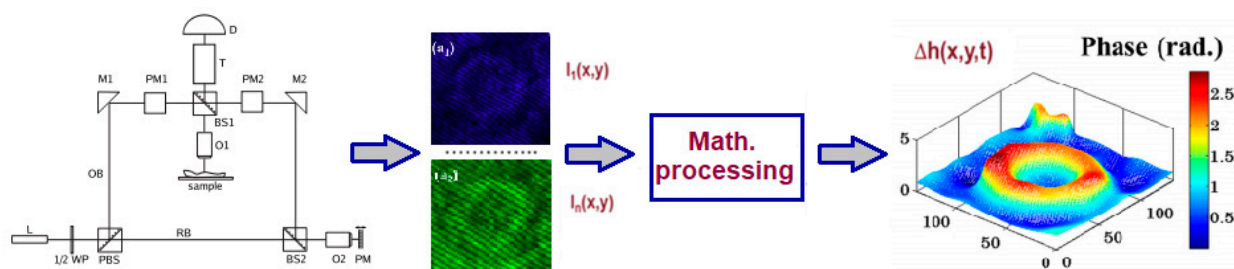


Figure 5. Scheme of obtaining a phase portrait of the investigated surface [102].

There are many strategies for effectively solving problems associated with the most accurate and reliable measurement of the thickness of a thin polymer coating [103]—a theoretical calculation or an empirical characteristic of the system on an opaque artifact on the surface or in the thickness of the film, providing a signal model representing the response of the instrument in the absence of a film. In [104], samples of chitosan films were studied with a phase-modulation laser interferometric microscope. A non-invasive assessment of the effect of the structure, composition and conditions of modification of the biopolymer composites on the viability, adhesive properties and functional activity of the living blood cells (neutrophils, lymphocytes and platelets) *in vitro* was carried out. A number of densitometry criteria have been proposed to identify the most promising biopolymer samples for the development of medical devices with characteristics as close as possible to physiological ones.

Obtaining quantitative values of the morphology of living cytoobjects makes it possible to track their change in real-time by obtaining 2D and 3D images. Due to the high speed of measurements and the non-invasiveness of this method, it has taken one of the central positions in the study of the tolerance of cellular objects to the surface of biomedical devices [99,104,105].

The generally accepted principle for studying the surface structure of various materials using microscopic methods is that more than one method should be used to obtain a complete picture of the surface whenever possible. The data obtained by two or more methods should not contradict each other but should complement each other and confirm the obtained characteristics of the test sample [93].

Thus, today, such microscopic methods as atomic force microscopy (AFM), scanning electron microscopy (SEM) and fluorescence and confocal microscopy [106–109] are known and described in the study of scaffolds based on chitosan and fibroin. The use of interference microscopy [99], tensiometry [110] and ellipsometry [111] are used to study adhesion and adsorption on the surface of the scaffolds. One of the most universal parameters for characterizing the surface and cell adhesion is the value of the surface energy of the material [95]. Features, possibilities and limitations of microscopic methods for studying the surface of biomaterials based on fibroin and chitosan and its energy characteristics are summarized in Table 1.

Its high resolution and ability to detect even the smallest objects (10 nm), as well as the ability to work in non-contact and semi-contact modes, makes AFM an excellent method for studying biological systems. In particular, a quantitative assessment of intermolecular interactions in biopolymer systems is of interest to many researchers. Understanding the molecular level of interfacial adhesion has great potential in areas such as the development of new dosage forms, biosensors and biomaterials [112,113].

Table 1. Research of the surface properties of biomaterials based on fibroin and chitosan.

Polymer Matrix Type, Reference	Composition of the Studied Sample	Surface Structure Research Method	Research Method Capabilities	Method Limitations
Aligned three-dimensional nanofibrous scaffolds [106]	silk fibroin-chitosan (eSFCS) (50:50)	atomic force microscopy (AFM)	Study of the three-dimensional geometry of the surface of the object under investigation with micro- and nanometer spatial resolution without special preparation and coloring. Study of morphofunctional features of individual cells. Characterization of the mechanical properties of the scaffold surface.	Risk of damage to the sample and distortion of the test result
Pore scaffold [107]	silk fibroin/collagen (SF/C) and silk fibroin/chitosan (SF/CS)	scanning electron microscopy (SEM)	High resolution for surface imaging. Study of the peculiarities of the interaction of cells with the material and the effect of tissue reaction on the implant. Analysis of the structure and interfaces between cells and matrix.	Invasive method. The need for sample preparation (like gold plating)
Scaffold with appropriate composition to mimic matrix of the damaged tissue with stem cells [108]	silk-fibroin (SF)/chitosan (CS)/(80:20)	fluorescence microscope	Used to assess the fluorescence intensity of stained cytoobjects. Allows monitoring the proliferative activity and migration of cells on the matrix surface in real-time. Obtaining quantitative data of aggregated statistical indicators for each type of cell and conclusions on the level of expression of cell markers. Descriptive statistics and comparison of levels for the expression of each cellular marker.	Invasive method. The need to prepare a sample by introducing a fluorescent label
Scaffold with open pore structure with desired pore size and porosity [109]	optimal silk fibroin–chitosan blend ratio of 80:20	confocal laser scanning microscopy	It differs from conventional fluorescence microscopy in non-invasiveness and improved resolution along the optical axis of the objective. It is used for morphological studies of the surface of cells and matrices, allowing one to obtain more informative data, presented immediately in digital form, which is suitable for quantitative and morphometric analysis. Possibility of studying immunocytochemical reactions with subsequent fluorescent detection of antibodies bound to the studied surfaces.	The need to prepare a sample by introducing a fluorescent label
Nanofiber scaffolds and films [99]	silk-fibroin (SF)/chitosan (CS) (25:75)	laser interferometry	Unlike other methods, it is non-invasive. Allows one to register and visualize information about the structure and properties of the surface in real-time. Reliable measurement of the thickness and roughness parameters of optically transparent biological and biopolymer objects (0.05–1000 µm). High-speed study of the morphological and functional features of individual cells with high resolution, with the possibility of obtaining 2D and 3D models.	The requirement for the transparency of biopolymers and cytoobjects.
Biodegradable scaffold that mimics the extracellular matrix (ECM) of host tissue [110]	silk fibroin/chitosan (SF/CS) (80:20)	water using tensiometer	Study of the behaviour of liquids, including water at the water/polymer material interface, to solve problems in the study of the surface properties of materials intended for contact with the environment of a living organism. Obtaining information on intermolecular forces and dynamics of interactions of macromolecular chains, hydrophobic and hydrophilic effect, adhesion on contact with a scaffold surface, sorption capacity of various low molecular weight substances, surface physicochemistry and surface phenomena for biopolymer matrix coatings of various types. Determination of the numerical value of the surface energy. Characterization of the surface physicochemistry and surface topography of biopolymer matrix coatings, the study of the effect of the polymer surface and its roughness on proliferation, differentiation and cell adhesion.	Certain requirements for the surface of the research object: the impossibility of studying large-pore objects.

Table 1. Cont.

Polymer Matrix Type, Reference	Composition of the Studied Sample	Surface Structure Research Method	Research Method Capabilities	Method Limitations
20-bilayer assemblies scaffold [111]	silk fibroin/collagen (SF/C)	ellipsometry	The possibility of simultaneous measurement of amplitude and phase characteristics makes it possible to accurately determine simultaneously the thicknesses of films and matrices and the optical constants of the material. High-precision determination of the roughness height and thickness (depth) (angstroms, nanometers, microns) and its dimensions in the interface plane. Characterization of the composition, conductivity and other properties of the material.	Difficulty in choosing the correct model of the reflecting system and interpreting the measurement results.

However, AFM and SEM methods are mainly used to obtain information on surface morphology. At the same time, for example, such properties of matrices as maintaining adhesion and proliferation of cells in cellular and tissue-engineered structures are determined by the three-dimensional morphology of the matrix; the spatial distribution of micropores, nanopores and nanoadditives in its volume; and nanostructural features of interfaces between matrices and cells. SEM does not allow examining biological (water-containing) samples without preliminary dehydration, which violates their initial structure, but it gives the clearest images of the surface morphology and sections of anhydrous samples of porous materials, in particular matrices for tissue engineering. The tensiometry method is used to study the surface properties of materials intended for contact with the environment of a living organism. It allows one to assess the effect of the polymer surface and its roughness on proliferation, differentiation and cell adhesion. The method is not suitable for studying large-porous objects. Confocal laser scanning microscopy is considered a highly accurate non-invasive method that allows one to register and visualize information about the structure and properties of the surface of biopolymer and biological objects in real-time. It is used for morphological studies of the surface of matrices when populating them with cells; it allows simultaneous visualization of both the matrix and the cells by using different fluorescent labels. Fluorescent microscopy is more or less damaging due to the use of contrasting or fluorescent dyes. For these reasons, novel techniques based on the measurement of cellular intrinsic optic properties have become increasingly popular. Interference microscopy has great opportunities. Application of phase-modulating laser interference microscopy has prospects for non-invasive imaging of unstained living cells on various biopolymer surfaces in dynamics.

7. Conclusions

Biomaterials play a critical role in the development of areas in tissue engineering, such as the reproduction of organs and tissues. Biodegradable polymers, especially those of natural origin, have proven themselves in the fabrication of scaffold systems to support life and cell activity. Topical issues and areas of research in the development of biopolymer scaffolds include the optimization of the physical, biological, chemical, mechanical and surface properties of scaffolds, depending on the environment of their application. Surface design and microstructuring for imitation of natural tissues with the implementation of biomimetic approaches, with the possibility of assessing surface properties at the interface, is one of the promising directions in tissue engineering.

Obtaining information about the structure and properties of the surface at the interface, as well as about its change in interaction with other objects of research, plays an important role in medicine and cell engineering. The method of processing chitosan and fibroin solutions sets the type of surface morphology of the biopolymer matrix, the roughness and porosity of which affects the surface wettability, adsorption of proteins and cell attachment and further growth, as well as the course of the inflammatory reaction. The surface charge of the matrix material and its hydrophilic–hydrophobic balance due to

interaction with the cell membrane affect the result of the interaction of the matrix with the cell membrane on cell adhesion and can be characterized by contact angles or surface energy. When choosing research methods, one should be guided by the morphological characteristics of biopolymer objects, water content, mechanical properties and resistance to impact on the object. Today, AFM is one of the leading microscopy methods in the field of materials science and tissue engineering due to its unique advantages and wide capabilities. Atomic force microscopy is the most informative for contact and semi-contact modes of use, which provide contact, albeit short-term, with the sample surface, and therefore, the use of these modes can distort the results of studying soft biological and biopolymer surfaces. Interference microscopy is a non-invasive research method; therefore, it is used to analyze biological objects and has prospects for studying the cytotoxicity of the surface of biopolymer materials and their biocompatibility with different types of surfaces. At the same time, interference microscopy has limitations associated with the dimensional characteristics and transparency of biopolymer materials. Comparative studies of the morphology of various types of biopolymer surfaces, their cytocompatibility using complementary minimally invasive methods—atomic force and interference microscopy—allow not only obtaining information about their morphofunctional properties but also formulating recommendations on the suitability of biopolymer objects for creating tissue-engineered constructs based on chitosan and fibroin.

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